

Appl. No. 10/071,349
Amdt. dated August 10, 2004
Reply to Office action of March 24, 2004

Amendments to the Specification:

Please replace paragraph [0166] with the following replacement paragraph:

[0166] The results from these experiments suggest, therefore, that the target (or targets) inhibited by the CD8⁺ suppressor molecule is (or are) one(s) which is (or are) active during the latest stages of the pseudotyped viruses virus life cycle subsequent to viral entry. Possible Primary targets therefore including include, but are not limited to, integration of viral DNA, transactivation from the proviral state, export of tat and/or rev into the cytoplasm and then back into the nucleus, and/or tat mediated enhancement of transcription

Please replace paragraph [0176] with the following replacement paragraph:

[0176] The present invention also provides a method for detecting a ~~CD8+ CD8⁺~~ suppressor molecule that has anti-HIV activity, the method comprising contacting a host cell with an env deficient HIV pseudotyped virus, comprising a reporter gene substituted for an HIV nef gene, such that said reporter gene is expressed in place of the HIV nef gene; contacting the host cell with a sample comprising (i) enriched ~~CD8+ CD8⁺~~ cells, (ii) a cell culture of ~~CD8+ CD8⁺~~ cells, or (iii) an extract or media component therefrom; and (c) measuring inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity.

Please replace paragraph [0181] with the following replacement paragraph:

[0181] The present invention also provides a diagnostic assay for monitoring clinical progression of HIV infection, said diagnostic assay comprising contacting a host cell with an env deficient HIV pseudotyped virus comprising a reporter gene substituted for an HIV nef gene such that said reporter gene is expressed in place of the HIV nef gene; contacting the host cell with samples from an HIV infection individual, said samples being collected successively from said individual; and measuring inhibition of reporter gene activity when each successive sample is contacted to the host cell, wherein a decrease in the inhibition inhibition of reporter gene activity when each successive sample is contacted to the host cell indicates progression of HIV infection.

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Please delete paragraph [0184].

Please replace paragraph [0187] with the following replacement paragraph:

[0187] In another embodiment, the viral entry inhibitor is an antibody that disrupts the interaction between a ~~CD4+ CD4⁺~~ cell surface receptor and a viral envelope protein. In a further embodiment, the antibody is a monoclonal antibody that specifically binds to the ~~CD4+ CD4⁺~~ receptor. In another embodiment, the particular stage of replication is reverse transcription.

Please replace paragraph [0191] with the following replacement paragraph:

[0191] The present invention also provides a method for obtaining a preparation containing a ~~CD8+ CD8⁺~~ suppressor molecule, said method comprising collecting conditioned media from cells expressing the ~~CD8+ CD8⁺~~ suppressor molecule; fractionating media components of said conditioned media; and identifying a fraction having ~~CD8+ CD8⁺~~ suppressor activity by a method comprising (i) contacting a host cell with a replication deficient pseudotyped HIV virus comprising a reporter gene operatively associated with an HIV promoter, (ii) contacting the host cell with a fractionated media component of said conditioned media, and (iii) measuring inhibition of reporter activity; wherein inhibition of reporter activity correlates with a fraction containing ~~CD8+ CD8⁺~~ suppressor activity. In a further embodiment, the reporter gene is expressed during early proviral gene expression.

Please replace paragraph [0192] with the following replacement paragraph:

[0192] The present invention also provides a method for obtaining a preparation containing a ~~CD8+ CD8⁺~~ suppressor molecule, said method comprising preparing an extract from a cell or cell line expressing the ~~CD8+ CD8⁺~~ suppressor molecule; fractionating components of said extract; and identifying a fraction having ~~CD8+ CD8⁺~~ suppressor activity by a method comprising (i) contacting a host cell with a replication deficient pseudotyped HIV virus comprising a reporter gene operatively associated with an HIV promoter, (ii) contacting the host cell with a fractionated component of said extract, and (iii) measuring inhibition of

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reporter activity; wherein inhibition of reporter activity correlates with a fraction containing a CD8+ CD8⁺ suppressor molecule. In a further embodiment, the reporter gene is expressed during early proviral gene expression.

Please replace paragraph [0194] with the following replacement paragraph:

[0194] In another embodiment, the pseudotyped virus is an env deficient pseudotyped virus. In a further embodiment, the pseudotyped virus is produced by a method which comprises co-transfected DNA for said pseudotyped virus with a vector that encodes a viral envelope protein. In one embodiment, the viral envelope protein is an HIV Env protein. In another embodiment, the viral envelope protein is a non-HIV envelope protein. In another embodiment, the conditioned media or extract is ~~prepared prepared~~ from a lymphocyte cell clone that expresses a CD8+ CD8⁺ suppressor molecule that inhibits HIV replication.

Please replace paragraph [0195] with the following replacement paragraph:

[0195] The present invention also provides a method for isolating a recombinant cDNA clone encoding a CD8+ CD8⁺ suppressor molecule that inhibits HIV replication, said method comprising constructing a cDNA expression library using mRNA prepared from CD8+ CD8⁺ T-lymphocytes that express a CD8+ CD8⁺ suppressor molecule; and screening cDNA products from said cDNA expression library using a method comprising, for each of said cDNA products, (i) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter, (ii) contacting the host cell with a sample comprising a cDNA product from the cDNA expression library, and (iii) measuring inhibition of reporter gene activity; wherein inhibition of reporter gene activity indicates that a recombinant cDNA clone encodes a suppressor molecule that inhibits HIV.

Please replace paragraph [0197] with the following replacement paragraph:

[0197] In another embodiment, the method further comprises, before said screening step, a step of enriching the cDNA library by eliminating clones that hybridize to cDNAs prepared from mRNA of lymphocytes that do not express a CD8+ CD8⁺ suppressor molecule.